

SHORT COMMUNICATION

Nephila female gigantism attained through post-maturity molting

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Abstract. *Nephila* are known for the greatest degrees of sexual size dimorphism among orb weaving spiders (Araneioidea) and thus among terrestrial animals. However, a meaningful quantification of the dimorphism is lacking and the proximate developmental mechanisms of female gigantism are poorly understood, being attributed solely to female delayed maturation. Here we show that females in the giant wood spider *Nephila pilipes* (Fabricius 1793) become giants through facultative post-maturity molting, a phenomenon resulting in female carapaces on average 4.27 times longer than males' (ranging from 3 to 6.4 times), and female mass averaging 125 times the male's (ranging from 28 to 502 times). Although the small males follow a typical developmental pathway and reach maturity with their final molt, the females mature at varying sizes and instars and then continue to grow by molting the entire exoskeleton except their genitals. The newly discovered phenomenon of additional, single-sex, adult, non-genital molting may represent a critical developmental adaptation that facilitates female gigantism in *Nephila* as a response to fecundity selection.

Keywords: Sexual size dimorphism, development, fecundity selection, spider, *Nephila pilipes*

Sexual size dimorphism (SSD) describes a morphological syndrome in which male and female sizes differ significantly within a species. Although well-known vertebrate cases exist where males are the larger sex, more dramatic sexual size differences are usually found in animals with female-biased SSD (Norman et al. 2002). A variety of selection pressures may account for the evolution of female-biased SSD; a commonly invoked explanation is fecundity selection, which postulates that increased female size enables greater egg production (Higgins 2002; Kuntner & Coddington 2009). However, in arthropods, evolution towards gigantic females acts against substantial developmental constraints, as arthropods can only grow by molting, which almost invariably ceases upon maturity (e.g., Stillwell & Davidowitz 2010; Foelix 2011).

Among terrestrial animals, the most extreme female gigantism is found in orb weaving spiders, clade Araneioidea (Foellmer & Moya-Laraño 2007). At least four independent phylogenetic origins of SSD are postulated, and these are predominantly cases of females becoming evolutionary giants (Hormiga et al. 2000). Classical cases of female gigantism have been reported in the family Nephilidae (Kuntner et al. 2008; Kuntner & Coddington 2009), in particular in the genus *Nephila* (Fig. 1a), although precise quantifications of sexual mass differences are mostly lacking. In addition, the proximate mechanisms of the developmental pathways responsible for extreme female gigantism are not well understood and mostly attributed to delayed maturation in females as a response to fecundity selection (Higgins & Goodnight 2010; Higgins et al. 2011). As most reports of SSD in spiders derive from scattered measurements of body length, and as body size is confounded with condition (Foellmer & Moya-Laraño 2007), body mass should instead serve as a better measure of SSD. Here, we report the range of female to male mass ratio as an accurate quantification of female gigantism and compare it to the carapace size ratio. In addition we report our discovery of a previously unknown developmental mechanism that underlies female gigantism in the giant wood spider *Nephila pilipes* (Fabricius 1793) (Fig. 1a).

We collected 155 adult males and 108 subadult females of *N. pilipes* on Pulau Ubin, Singapore (1.421575°N, 103.932542°E). In the

laboratory, we reared the males in plastic cups and the subadult and adult females in their own webs made in frames. We fed the males fruit flies, and the females flies and mealworms thrice a week, and watered them daily.

In most spiders molting ceases with maturity, and this has also been conventionally presumed in *Nephila* and other araneoid spiders (for exceptions, see below). However, in our pool of 40 subadult females that were subjected to daily monitoring until their maturity, 27 (67.5%) molted even after maturity (mean number of molts = 1.15, range = 1–2; SD = 0.36, $n = 27$). Our morphological examination of 17 molts (or exuvia) that were shed after maturity revealed no molted genital structures. Each exuvium contained a hole in the part of the ventral abdomen that contains the genitals (Fig. 1b). No adult female molted after egg sac and plug formation (Kuntner et al. 2012).

Adult female carapace length ranged between 7.09 and 11.52 mm (mean = 8.80 mm, SD = 0.90 mm, $n = 31$), and that of males ranged between 1.80 and 2.36 mm (mean = 2.06 mm, SD = 0.15 mm, $n = 50$). Adult females weighed between 0.385 and 1.757 g (mean = 0.930, SD = 0.375, $n = 31$), and adult males between 0.0035 and 0.0137 g (mean = 0.0074, SD = 0.002, $n = 98$). In our population, females were on average 4.27 times larger than males in carapace length (ranging from 3 to 6.4), but were 125 times heavier than the males (with extremes ranging from 28 to 502 times male mass). According to the review of Foellmer & Moya-Laraño (2007), our SSD quantification in *N. pilipes* reflects the most extreme mass dimorphism reported in a terrestrial animal (for an extreme among marine animals, however, see Norman et al. 2002).

Post-maturity molting was more likely in females that copulated for a shorter total duration (Mann-Whitney $U = 81.5$, $n = 39$, $P = 0.023$), and, albeit non-significant, such a trend was also present in females exposed to lower levels of polyandry and fewer copulatory insertions (Mann-Whitney $U = 100$, $n = 39$, $P = 0.088$; number of males: $\chi^2 = 7.12$, $df = 3$, $P = 0.068$). This suggests that females, regardless of their size and mass, may terminate their growth after possessing enough sperm for oviposition, but that they may continue to grow and molt if the number or duration of matings has been below a certain threshold. It is important to note that molting females

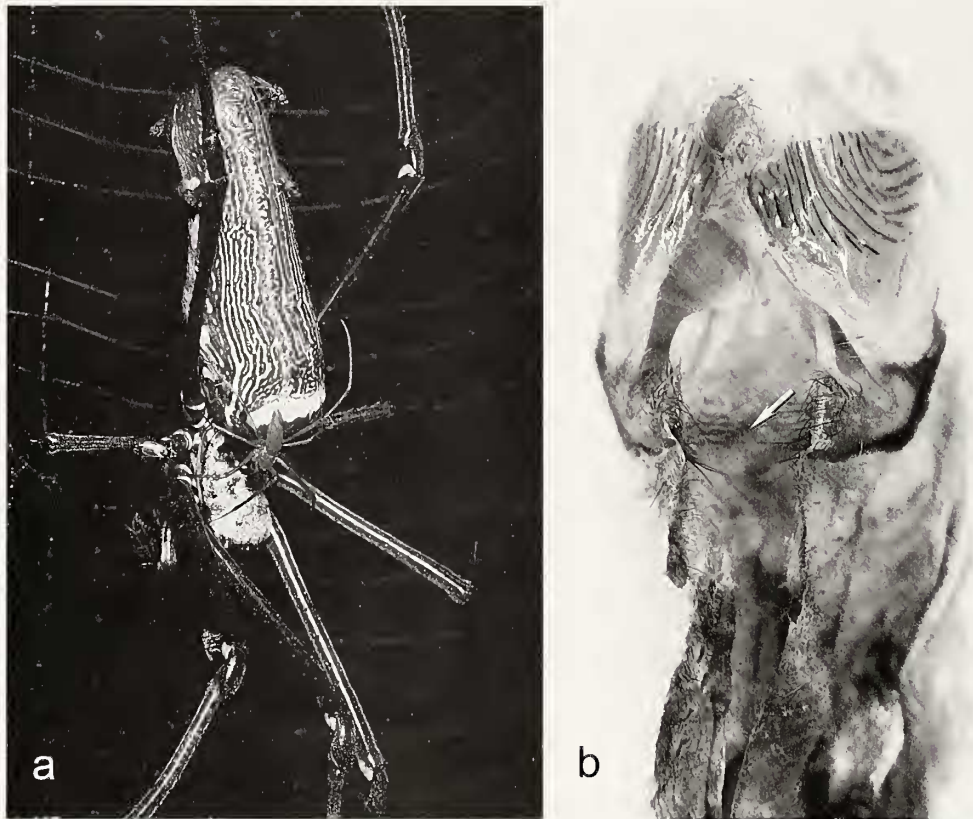


Figure 1a, b.—The giant wood spider *Nephila pilipes*, a highly polygamous and sexually dimorphic species whose females are on average 125 times heavier than males. a. A gravid and fully grown female in nature supporting on her body a conspecific male and six kleptoparasitic flies. b. Female adult molt containing the entire abdominal exoskeleton except the genital area (arrow).

do not shed their genitals and thus may retain sperm already stored. Such developmental plasticity may be a critical response to fecundity selection, which in *Nephila* results in extreme female gigantism. As reported here, females are freed from developmental constraints to be capable of growth beyond maturity and presumably increased egg production. Other explanations are possible, however. For example, chemical signaling by a molted female may attract more males, which may serve the female as she strives for polyandry (Kuntner et al. 2012). In support of this may be the case of *Nephila clavata*, where males are highly attracted to recently molted females (Miyashita & Hayashi 1996).

Our discovery of post-maturity female growth in *Nephila pilipes* is at odds with the conventional wisdom that most spiders exhibit determinant growth and do not molt after maturity (Foelix 2011). Post-maturity molting has predominantly been known in phylogenetically more ancestral and sexually monomorphic spiders such as liphistiids (Haupt 2003; Foelix 2011) and mygalomorphs (Baerg 1958; Miyashita 1992; Schmidt 1993); in these groups of long lived spiders, adult female molting involves growth by shedding the entire exoskeleton, including the genitals (Schmidt 1993; Foelix 2011). However, studies have also outlined cases of post-maturity molting in phylogenetically scattered examples of araneomorph spiders, such as social eresids, genus *Stegodyphus* (Kraus and Kraus 1988), female filistatids (Vetter 2011) and male *Loxosecles*, where, apparently, eunuch males engage in fatal post-maturity molting attempts (Vetter 2011). There exist some other reports for araneomorphs, but most are exceptional or anomalous (Fujii 2001; see also Kayashima 1981). For example, as the only known case of post-maturity molting in araneoids, Kaston (1968) reports it happened in five out of hundreds of black widow females (*Latrodectus mactans* and *L. hesperus*), and those molts, as in *Nephila*, did not contain any epigynal structures; molted females in fact retained enough sperm in their spermathecae to

lay fertile eggs post molting. However, the rarity of post-maturity molting in *Latrodectus*, albeit suggestive of a potentially more widespread ability of araneomorph spider adults to molt, nevertheless suggests that it is not an obligate, but rather an anomalous life history trait (Kaston 1968).

Because other araneoid spiders do not usually molt as adults, the mechanism of non-genital molting of adult *Nephila* females may represent a critical developmental adaptation facilitating gigantism, and involves the additional dimension of sexual dimorphism. Although *N. pilipes* males follow typical spider developmental pathways and reach maturity with their final molt, females mature at varying sizes and then continue to grow by molting the entire exoskeleton except their genitals. However, it remains to be established how typical such development is for *Nephila*, or even if it is prevalent in all populations of *N. pilipes*, a widespread species ranging throughout South, Southeast and East Asia into Australasia (Su et al. 2007), and thus from rain forests to dry subtropical forests. Post-maturity molting was apparently unknown to Harvey et al. (2007), who revised Australasian *Nephila*. In their detailed studies of the Papuan population of *N. pilipes* (as *N. maculata*), Robinson & Robinson (1973, 1976) postulated 14 instars in the female and 7 in the male. They acknowledged some confusion as to the number of instars, but nevertheless only considered the last, 14th instar, to represent an adult female. Similarly, in a developmental study, Higgins (2002) did not report that several large instars in females from Papua might represent adult, not subadult females, although she concluded that males only go through four and females through about 10 instars. In our prior mating study on the Singapore population, we also failed to take this into account (Kuntner et al. 2009). We believe that instars 12 to 14 in the study of Robinson & Robinson (1976) represented adult females of different sizes, and that such plastic development will be typical of all populations of *N. pilipes*. In fact, we predict it also to be

the case in its sister species, the African *N. constricta* (Kuntner et al. 2008; Su et al. 2011), which shows an unprecedented range of adult female sizes (Higgins et al. 2011).

Biology of the giant wood spider *Nephila pilipes* never ceases to excite, as it is now known to encompass the largest documented mass difference between sexes among terrestrial organisms, with females on average 125 times heavier than males. Furthermore, a previously unstudied developmental mechanism of additional adult growth through molting exists, in addition to a plethora of behavioral adaptations arising through sexual selection that we believe are connected with, if not determined by, extreme SSD (Kuntner et al. 2009a). Notable examples of such behaviors are extreme polygamy, mating plugs, genital mutilation, mate binding (Kuntner et al. 2009b, 2012; Zhang et al. 2011) and the construction of giant asymmetrical webs (Kuntner et al. 2010).

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LITERATURE CITED

- Baerg, W.J. 1958. The tarantula. University of Kansas Press, Lawrence, Kansas; Fitzgerald Publishing, London, UK.
- Foelix, R.F. 2011. Biology of Spiders, 3rd edition. Oxford University Press, Oxford, UK.
- Foellmer, M.W. & J. Moya-Laraño. 2007. Sexual size dimorphism in spiders: patterns and processes. Pp. 71–81. *In*: Sex, size and gender roles: Evolutionary studies of sexual size dimorphism. (D.J. Fairbairn, W.U. Blanckenhorn & T. Székely, eds.). Oxford University Press, New York.
- Fujii, Y. 2001. Post-maturation molt found in a wolf spider, *Pardosa astrigera* (Araneae, Lycosidae). *Journal of Arachnology* 29:263–266.
- Harvey, M.S., A.D. Austin & M. Adams. 2007. The systematics and biology of the spider genus *Nephila* (Araneae: Nephilidae) in the Australasian region. *Invertebrate Systematics* 21:407–451.
- Haupt, J. 2003. The Mesothelae – a monograph of an exceptional group of spiders (Araneae: Mesothelae). *Zoologica* 154:1–102.
- Higgins, L. 2002. Female gigantism in a New Guinea population of the spider *Nephila maculata*. *Oikos* 99:377–385.
- Higgins, L., J.A. Coddington, C. Goodnight & M. Kuntner. 2011. Testing ecological and developmental hypotheses of mean and variation in adult size in nephilid orb-weaving spiders. *Evolutionary Ecology* 25:1289–1306.
- Higgins, L. & C. Goodnight. 2010. *Nephila clavipes* females have accelerating dietary requirements. *Journal of Arachnology* 38: 150–152.
- Hormiga, G., N. Scharff & J.A. Coddington. 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders (Araneae, Orbiculariae). *Systematic Biology* 49:435–462.
- Kaston, B.J. 1968. Remarks on black widow spiders, with an account of some anomalies. *Entomological News* 79:113–124.
- Kayashima, I. 1981. A report on long-term rearing of *Heteropoda venatoria* (Linne) (1). *Kishidaia* 47:57–64.
- Kraus, O. & M. Kraus. 1988. The genus *Stegodyphus* (Arachnida, Araneae). Sibling species, species groups and parallel origin of social living. *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 30:151–254.
- Kuntner, M. & J.A. Coddington. 2009. Discovery of the largest orbweaving spider species: The evolution of gigantism in *Nephila*. *PLoS ONE* 4(10):e7516.
- Kuntner, M., J.A. Coddington & G. Hormiga. 2008. Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics* 24:147–217.
- Kuntner, M., J.A. Coddington & J.M. Schneider. 2009a. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). *Evolution* 63:1451–1463.
- Kuntner, M., M. Gregorič & D. Li. 2010. Mass predicts web asymmetry in *Nephila* spiders. *Naturwissenschaften* 97:1097–1105.
- Kuntner, M., M. Gregorič, S. Zhang, S. Kralj-Fišer & D. Li. 2012. Mating plugs in polyandrous giants: Which sex produces them, when, how and why? *PLoS ONE* 7(7):e40939 2010.
- Kuntner, M., S. Kralj-Fišer, J.M. Schneider & D. Li. 2009b. Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis. *Journal of Zoology* 277:257–266.
- Miyashita, K. 1992. Postembryonic development and life cycle of *Atypus karschi* Doenitz (Araneae: Atypidae). *Acta Arachnologica* 41:177–186.
- Miyashita, T. & H. Hayashi. 1996. Volatile chemical cue elicits mating behavior of cohabiting males of *Nephila clavata* (Araneae, Tetragnathidae). *Journal of Arachnology* 24:9–15.
- Norman, M.D., D. Paul, J. Finn & T. Tregenza. 2002. First encounter with a live male blanket octopus: the world's most sexually size-dimorphic large animal. *New Zealand Journal of Marine and Freshwater Research* 36:733–736.
- Robinson, M.H. & B. Robinson. 1973. Ecology and behavior of the giant wood spider *Nephila maculata* (Fabr.) in New Guinea. *Smithsonian Contributions to Zoology* 149:1–73.
- Robinson, M.H. & B. Robinson. 1976. The ecology and behavior of *Nephila maculata*: a supplement. *Smithsonian Contributions to Zoology* 218:1–22.
- Schmidt, G. 1993. *Vogelspinnen*, 4th edition. Landbuch-Verlag GmbH, Hannover, Germany.
- Stillwell, R.C. & G. Davidowitz. 2010. A developmental perspective on the evolution of sexual size dimorphism of a moth. *Proceedings of the Royal Society London B: Biological Sciences* 277:2069–2074.
- Su, Y.C., Y.H. Chang, D. Smith, M.S. Zhu, M. Kuntner & I.M. Tso. 2011. Biogeography and speciation patterns of the golden orb spider genus *Nephila* (Araneae: Nephilidae) in Asia. *Zoological Science* 28:47–55.
- Su, Y.C., Y.H. Chang, S.C. Lee & I.M. Tso. 2007. Phylogeography of the giant wood spider (*Nephila pilipes*, Araneae) from Asian-Australian regions. *Journal of Biogeography* 34:177–191.
- Vetter, R.S. 2011. Unique eunuchs? Fatal post-maturity molting in male *Loxosceles laeta* (Nicolet 1849) (Araneae: Sicariidae) after losing both palps. *Pan-Pacific Entomologist* 87:138–144.
- Zhang, S., M. Kuntner & D. Li. 2011. Mate binding: male adaptation to sexual conflict in the golden orb-web spider (Nephilidae: *Nephila pilipes*). *Animal Behaviour* 82:1299–1304.

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